IN THE NAME OF GOD

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Research Communication

Aberrant Methylation of *APC* and *RARβ2* Genes in Breast Cancer Patients

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2014 ; in Egypt

Estimated Deaths

Males			Females		
Lung & bronchus	85,920	27%	Lung & bronchus	72,160	26%
Prostate	26,120	8%	Breast	40,450	14%
Colon & rectum	26,020	8%	Colon & rectum	23,170	8%
Pancreas	21,450	7%	Pancreas	20,330	7%
Liver & intrahepatic bile duct	18,280	6%	Ovary	14,240	5%
Leukemia	14,130	4%	Uterine corpus	10,470	4%
Esophagus	12,720	4%	Leukemia	10,270	4%
Urinary bladder	11,820	4%	Liver & intrahepatic bile duct	8,890	3%
Non-Hodgkin lymphoma	11,520	4%	Non-Hodgkin lymphoma	8,630	3%
Brain & other nervous system	9,440	3%	Brain & other nervous system	6,610	2%
All Sites	**314,290**	**100%**	**All Sites**	**281,400**	**100%**

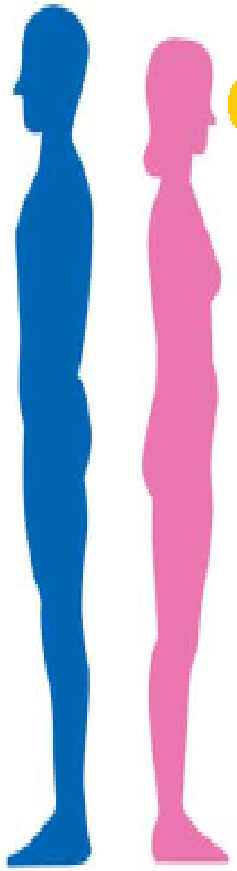
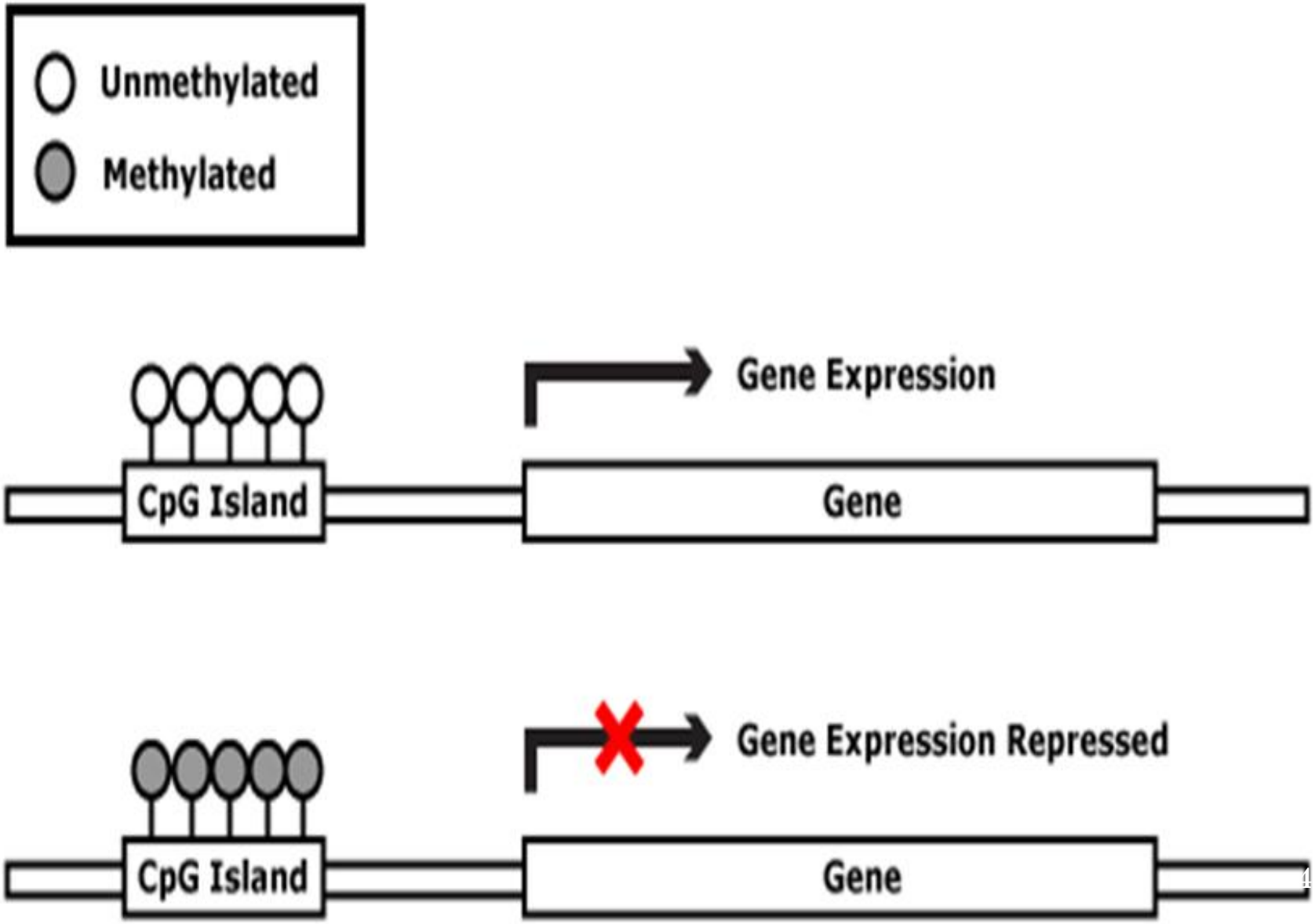


FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2016.
Estimates are rounded to the nearest 10 and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.


Unmethylated
Methylated
Gene Expression
CpG Island
Gene
Gene Expression Repressed
CpG Island
Gene

- Samples :

- 121 malignant breast cancer (71/121 Triple negative)
- 79 benign Lesion
- 66 Healthy control

alternative mechanism for inactivation of tumor suppressor genes (5). Because gene hypermethylation has been found to be a common and early alteration in many tumor types (6), including breast (7), it has emerged as a promising target for detection of breast cancer. Several tumor suppressor genes have been found to be hypermethylated in breast cancer and unmethylated in normal cells, *e.g., adenomatous polyposis coli* (*APC*) (8), and *retinoic acid receptors-β_2 (RAR-β_2)* (9).

Detection of methylated DNA in the serum of cancer patients is quite intriguing and has been suggested to be a marker for early cancer development (10). Their concentration in cancer patients is higher than that from healthy people (11). Methylation specific-polymerase chain reaction (PCR) (MSP) a technique that can be used to detect hypermethylations as it can identify up to 1 methylated allele in 1000 unmethylated alleles, appropriate for the detection of neoplastic cells in a background of normal cells (12). It has been used in recent studies for the successful detection of cancer cell DNA in body fluids such as liver (13), lung (14), and head and neck cancer in serum (15), lung cancer in both sputum (16) and bronchial lavage (17), and bladder cancer in urine (18,19). Overall, these studies have demonstrated a high concordance between the epigenetic alterations found in primary tumor specimens and in body fluids, suggesting the potential utility of these alterations as surrogate molecular markers. Thus far, however,

teers. After obtaining informed co[illegible]m participants for the use of blood in this study, all o[illegible] them [illegible]ere interviewed to elicit information regarding demo[illegible]raphic [illegible]haracteristics; full clinical and pathological data [illegible]re obtai[illegible]d from medical reports.

Collection and Processing of Samples and Assessment of Classical Tumor Markers

At the enrollment visit, 5 ml of blood was drawn into tube and then centrifuged at 1600*g* for 15 min at 15–20°C. The serum was stored at −80°C until processing for assessment of classical tumor markers and DNA extraction. Classical tumor markers (CEA and CA 15.3) were done by solid phase enzyme linked immunosorbent assay (Signosis, Sunnyvale).

Detection of Methylated RARβ_2 and APC Genes

This was accomplished in four steps. In the first step, DNA was extracted from serum samples. In the second step, DNA was treated by bisulfite. Third step, the gene was amplified by PCR. Finally, the fourth step included detection of the PCR products using agarose gel.

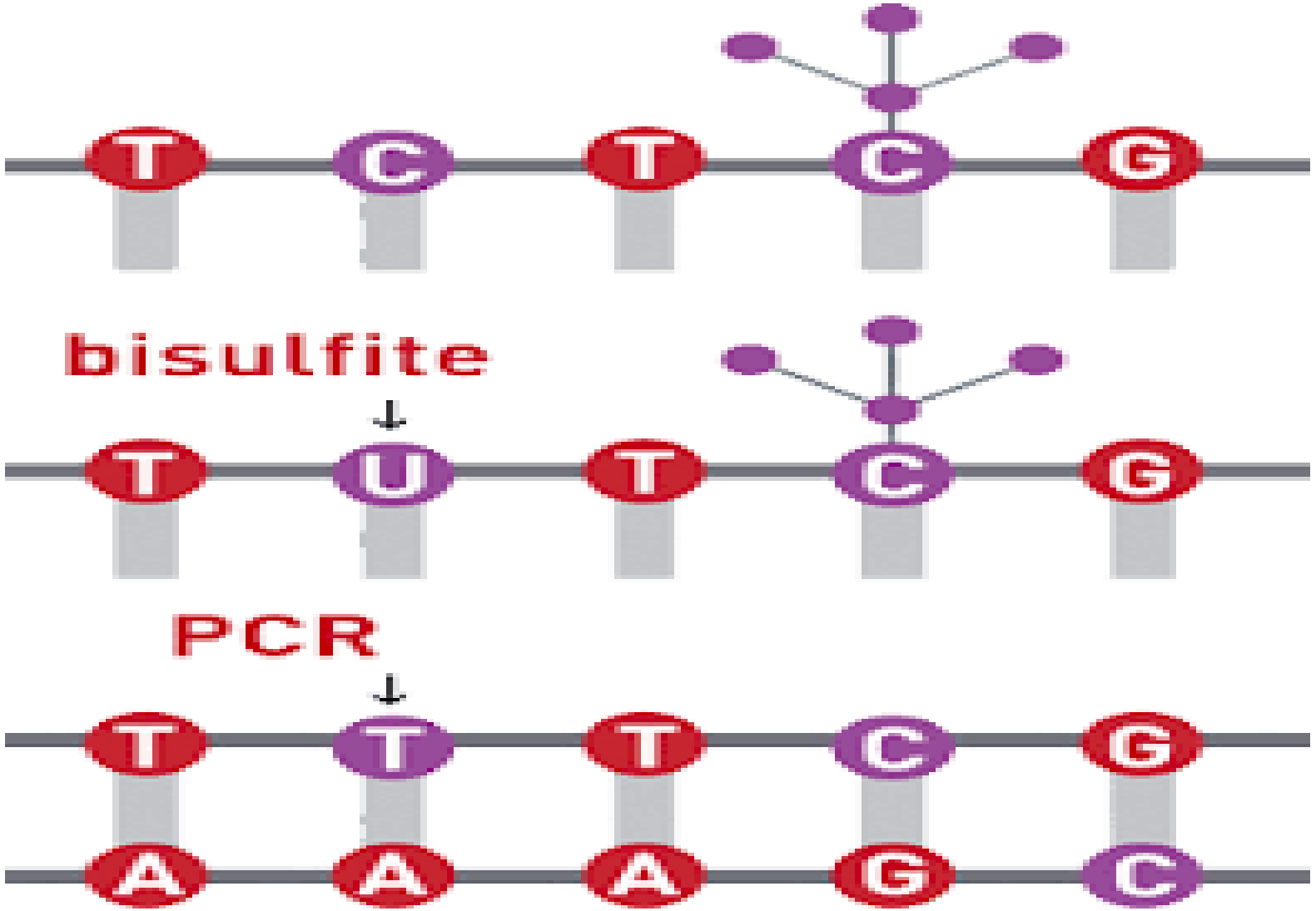
1. DNA isolation from serum samples

   DNA extraction was done using QIAamp DNA Mini blood kit (QIAGEN, USA). In brief, 200 µl serum was added with 20 µl QIAGEN protease into microcentrifuge tube, samples were

- **Detection of Methylated RARB2 and APC Genes** was accomplished in four steps:

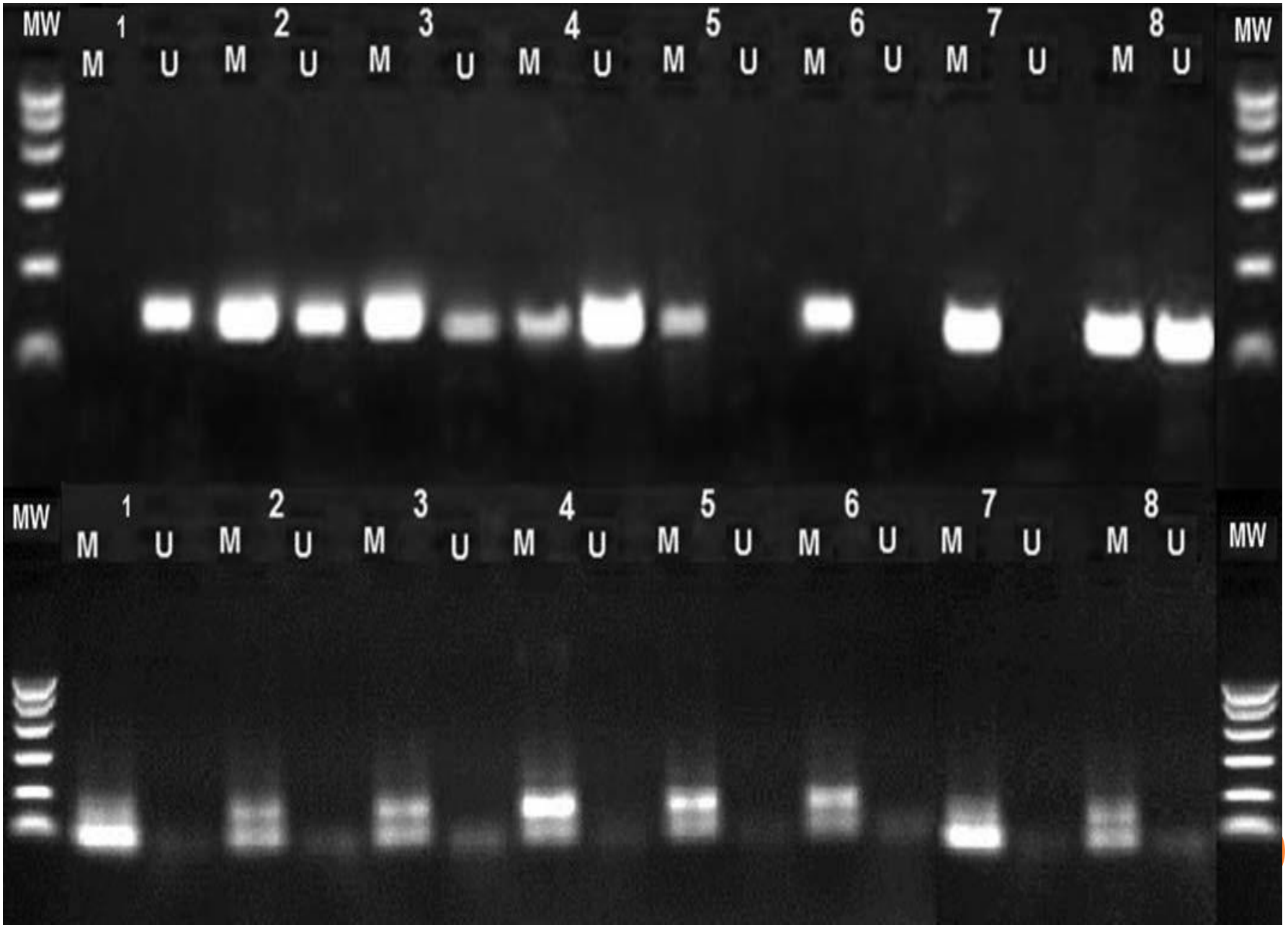
1. DNA Extraction (by use of QIAamp DNA Mini blood Kit)
2. Treat by bisulfite
3. Amplified by PCR
4. Gel Electrophoresis

Sodium bisulfate treatment



- PCR condition in 50 µl volume of reaction

- Activation at 95°C for 5 min ; then 35cycles
- Denaturation at 94°C for 1 min
- Annealing 1 min ; For APC at 53 °C
  For RARB2 at 50 °C
  For hMLH1 at 58 °C
- Extension at 72°C for 1 min
- Final extension at 72°C for 10 min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Patient's characteristics	*Cases (n = 121)*
Age (years)	
≤42	54 (44.6%)
>42	67 (45.4%)
Menopausal status	
Premenopausal	94 (77.7%)
Postmenopausal	27 (22.3%)
ER-status	
Negative	46 (38%)
Positive	75 (62%)
PgR-status	
Negative	61 (50.4%)
Positive	60 (49.6%)
HER-2/neu status	
Negative	45 (44.6%)
Positive	67 (55.4%)
Clinical stage	
Early stage	86 (71%)
Late stage	35 (29%)
Histological grade	
Low grade	89 (73.6%)
High grade	32 (26.4%)

TABLE 2 *APC and RARβ_2 methylation pattern and positivity levels of tumor markers (CEA and CA15.3) among different investigated groups*

Investigated markers	*Control (n = 66)*	*Benign (n = 76)*	*Malignant (n = 121)*
Methylated *APC* (*n* = 119)	0 (0%)	6 (7.8%)	113 (93.4%)
Homoplasmic methylation (*n* = 109)	0 (0%)	0 (0%)	109 (90.1)
Heteroplasmic methylation (*n* = 10)	0 (0%)	6 (7.8%)	4 (3.3%)
Methylated *RARβ_2* (*n* = 127)	0 (0%)	11 (14.5%)	116 (95.9%)
Homoplasmic methylation (*n* = 113)	0 (0%)	9 (11.8%)	104 (86%)
Heteroplasmic methylation (*n* = 14)	0 (0%)	2 (2.6%)	12 (9.9%)
Positive CEA (>5 ng/ml)	0 (0%)	34 (44.7%)	63 (52.1%)
Positive CA15.3 (>15 ng/ml)	0 (0%)	20 (26.3%)	91 (75.2%)

Patient's characteristics	*Methylated RARβ$_2$*	*Methylated APC*	*Positive CEA*	*Positive CA15.3*
Age (years)				
≤42	51 (94.4%)	51 (94.4%)	33 (61.1%)	43 (79.6%)
>42	65 (97%)	62 (92.5%)	30 (44.8)	48 (71.6%)
Menopausal status				
Premenopausal	91 (96.8%)	87 (92.6%)	52 (55.3%)	74 (78.7%)
Postmenopausal	25 (92.6%)	26 (96.3%)	11 (40.7%)	17 (63%)
TNB				
Cases with TNB	71 (100%)	67 (94.4%)	34 (47.9%)	53 (74.6%)
Cases with non-TNB	45 (90%)	46 (92.2%)	29 (58%)	38 (76%)

$\chi^2 = 7.4, P = 0.007$

	Breast cancer		Early stage		Low grade		Triple negative breast cancer	
Investigated items	*Sens.%*	*Spec.%*	*Sens.%*	*Spec.%*	*Sens.%*	*Spec.%*	*Sens.%*	*Spec.%*
APC promoter hypermethylation	93.4	95.4	94.2	95.9	94.4	95.9	92	94.4
RARβ$_2$ promoter hypermethylation	95.5	92.4	95.3	92.4	95.5	92.4	90	100
CEA	52.1	76.7	55.8	76.6	53.9	76.6	58	74.9
CA15.3	75.2	86.2	76.7	86.2	74.2	86.2	76	74.6


Thank You